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Agace, William Winston; McCoy, Kathy D.

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Regionalized Development and Maintenance of the Intestinal Adaptive Immune Landscape

William W. Agace^{1,2,*} and Kathy D. McCoy^{3,*}

¹Division of Immunology and Vaccinology, National Veterinary Institute, Technical University of Denmark (DTU), 2800 Kongens Lyngby, Denmark

²Immunology Section, Department of Experimental Medical Science, Lund University, BMC D14, Sölvegatan 19, 221 84 Lund, Sweden

³Department of Physiology and Pharmacology and Calvin, Phoebe and Joan Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada

*Correspondence: wia@vet.dtu.dk (W.W.A.), kathy.mccoy@ucalgary.ca (K.D.M.)

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The intestinal immune system has the daunting task of protecting us from pathogenic insults while limiting inflammatory responses against the resident commensal microbiota and providing tolerance to food antigens. This role is particularly impressive when one considers the vast mucosal surface and changing landscape that the intestinal immune system must monitor. In this review, we highlight regional differences in the development and composition of the adaptive immune landscape of the intestine and the impact of local intrinsic and environmental factors that shape this process. To conclude, we review the evidence for a critical window of opportunity for early-life exposures that affect immune development and alter disease susceptibility later in life.

Introduction

The intestine is a long tube-like structure stretching from the oral cavity to the anus, and its primary functions are to digest food, absorb nutrients and water, and eliminate waste. The intestine also represents a major site of entry for many bacterial and viral pathogens and is home to a vast and diverse microbial community increasingly recognized to have a major impact on host physiology and pathophysiology. This constantly changing and dynamic environment represents a key challenge to the immune system. It is thus perhaps unsurprising that the intestine contains the greatest number and diversity of immune cells in the body.

The intestine should not be viewed as a single homogeneous organ but rather as consisting of several anatomically and functionally specialized segments with distinct environmental pressures (for a review, see [Mowat and Agace, 2014](#)). To maintain local tissue homeostasis, different intestinal segments have adopted unique defense strategies, including alterations in epithelial subset composition and function, mucus layer integrity, and regionalized immune system specialization. Continual crosstalk between environmental signals and the immune system is essential for maintaining local tissue homeostasis, and alterations in such signals (e.g., from nutritional deficiencies or alterations in microbial composition [dysbiosis]) can rewire immune cell composition and functionality, resulting in chronic inflammation and increased risk for infection.

Here, we highlight the contribution of luminal signals in shaping and maintaining regional adaptive immune cell composition and function in the intestine. We further discuss the early environmental factors affecting intestinal immune system development and their long-term impact on health. Collectively, such studies highlight the importance of the environment in maintaining local immune homeostasis and the potential of modulating such signals in early-life and inflammatory settings for improving human health.

Regional Differences in Intestinal Anatomy, Function, and Luminal Content

The small intestine, whose primary function is in nutrient digestion and absorption, starts at the pylorus directly downstream of the stomach and terminates at the ileocaecal valve. In humans, it reaches 6–7 m in length and consists of the duodenum, jejunum, and ileum in descending order. The surface is characterized by long finger-like projections called villi, which become progressively shorter and broader down the small intestine. Villi are surrounded by intestinal crypt invaginations, termed the crypts of Lieberkühn, that contain the multi-potent epithelial stem cells that give rise to the various epithelial lineages covering the intestinal surface. The base of these crypts, particularly in the ileum, is home to specialized epithelial cells, termed Paneth cells, that produce a range of antimicrobial peptides that serve to protect the sterility of the crypt, as well as epithelial growth factors that regulate stem cell differentiation and function. Absorptive epithelial cells, termed enterocytes, coat the villi; their apical surface is covered with microvilli that form a “brush border” for optimal digestion and absorption of foodstuffs. In addition to the luminal content from the stomach, the duodenum receives 0.6–1.0 L of bile per day through the common bile duct. The major constituents of bile include cholesterol, lecithin, bilirubin, and bile salts, the latter of which provide a key detergent action on fat particles in the food and aid in the adsorption of fatty acids, monoglycerides, cholesterol, and other lipids. Absorption of mono-, di-, and tri-saccharides, amino acids, dietary fat, and fat (vitamins A, D, E, and K)- or water (vitamins B and C)-soluble vitamins occurs primarily in the duodenum and jejunum, whereas the ileum serves as a major site of bile salt and vitamin B12 absorption. Because many of these luminal constituents have immune-modulatory activity, their varying concentrations along the length of the intestine are likely to have an important impact on regulating regionalized immune cell compartmentalization and functionality.

The ileocecal valve opens into the large intestine, which (in humans) is composed of the caecum (and associated blind-ended

appendix), the ascending, transverse, descending, and sigmoid colon, and the rectum, and it terminates at the anal canal. The human colon is considerably shorter (approximately 1.5 m) and wider than the small intestine. Although also covered by a single layer of columnar epithelial cells, its surface consists entirely of crypts interspersed between flat regions of surface epithelium. Mucus-producing goblet cells make up a relatively small percentage ($\leq 10\%$) of epithelial cells in the small intestine but represent $\geq 25\%$ of epithelial cells in the large intestine. As a result, the mucous layer, termed the glycocalyx, is diffuse and permeable to bacteria in the small intestine but forms a thick bi-layered structure in the colon, whose inner layer is virtually impenetrable to bacteria. Although the majority of dietary constituents are absorbed in the small intestine, approximately 1.5 L of intestinal fluid passes through the ileocecal valve each day. This fluid consists primarily of water and electrolytes, most of which are absorbed in the upper half of the colon, as well as semi- or undigested foodstuffs, including plant polysaccharides and fiber, which serve as an essential nutritional source for resident microbiota.

The intestinal microbiome plays an essential role in host health and, particularly in the context of this review, in modulating local adaptive immune cell development, composition, and function. Microbial density increases from the upper to the lower gastrointestinal tract until it reaches its peak in the colon, which contains the highest microbial diversity and load. The mechanisms by which the microbiota modulates the intestinal immune system is under intense investigation but include (1) direct microbial interactions with host cells and (2) bacterial generation of immune modulatory molecules either through modification of dietary metabolites or through de novo production. The relative impact of these pathways in modulating immune homeostasis will vary along the length of the intestine depending on local variations in surface physiology (e.g., mucus integrity), microbial load, and microbial diversity.

Regionalized Variation in Intestinal Inductive Compartments

The intestine is composed of four major layers: the mucosa, the submucosa, the muscularis, and the serosa. The mucosa, which is proximal to the intestinal lumen, consists of a single layer of columnar epithelial cells and an underlying lamina propria (LP) and contains the vast majority of immune cells. Immune cells are located within organized lymphoid structures, termed gut-associated lymphoid tissues (GALTs), embedded within the intestinal LP and sub-mucosa or are diffusely distributed throughout the epithelium and LP. GALTs, together with intestinal draining lymph nodes (LNs), serve as the major sites of adaptive immune cell priming in the intestine. GALTs include the macroscopically visible Peyer's patches (PPs) of the small intestine, caecal patches, colonic patches, and smaller structures collectively referred to as solitary isolated lymphoid tissues (SILTs), which include cryptopatches (CPs) and more mature isolated lymphoid follicles (ILFs) (Hamada et al., 2002; Pabst et al., 2005). In humans, PPs are primarily located in the terminal ileum; their numbers peak in the midteens (approximately 250) and then wane with age (Cornes, 1965). Additional large GALT structures are found throughout the appendix. Although CPs have not been identified in the human intestine, there are an esti-

mated 30,000 ILFs, whose highest densities are in the ileum and distal colon (Moghaddami et al., 1998; O'Leary and Sweeney, 1986). Large GALT structures in mice include PPs, which are more numerous in the distal small intestine, a single large GALT in the caecum (the caecal patch), and two to five colonic patches (Baptista et al., 2013; Masahata et al., 2014). Mice also have 1,000–1,500 SILTs distributed throughout the small intestine and mature SILTs concentrated in the ileum, consistent with the finding that SILT maturation in the small intestine requires signals from the microbiota (Herbrand et al., 2008). Although SILT numbers in the colon have yet to be enumerated, they appear more abundant in the distal colon (Baptista et al., 2013).

GALTs lack afferent lymphatics and sample luminal particulate antigens through specialized microfold (M) cells located within an overlying follicular-associated epithelium. Antigens sampled through M cells are subsequently taken up by antigen-presenting cells in the underlying sub-epithelial dome of GALTs for processing and presentation to lymphocytes. In contrast, adaptive immune cell priming in intestinal draining LNs involves the transport and presentation of luminal or self-derived antigen by LP-derived migratory dendritic cells (DCs) (Macpherson and Uhr, 2004). Several mechanisms by which DCs acquire luminal antigen in the LP have been proposed, including (1) direct antigenic uptake via goblet-cell-associated antigen passages (McDole et al., 2012) or trans-epithelial extended DC-derived dendrites (Farache et al., 2013; Rescigno et al., 2001) or (2) indirect uptake via tissue-resident macrophages (Mazzini et al., 2014), apoptotic epithelial cells (Huang et al., 2000), or villous M cells (Jang et al., 2004). The relative contribution of these pathways in adaptive immune cell priming, and whether differential antigen uptake results in distinct adaptive immune responses, remains unclear.

Development of the Inductive Sites in the Intestine

The development of PPs is initiated during fetal life and is guided by intrinsic and programmed events, although maturation is completed postnatally and is influenced by external factors. In contrast, CPs and ILFs develop and mature after birth. In addition, the development of GALT structures in the small intestine and colon is guided by different signals.

In mice, the development of PPs and colonic patches is initiated on embryonic day 12.5 (Adachi et al., 1998). For PPs, the primary event is initiated by $CD45^+CD4^-CD3^-CD127^-cKit^+CD11c^+$ lymphoid tissue initiator (LTin) cells expressing $LT\beta$ and RET (Fukuyama and Kiyono, 2007; Veiga-Fernandes et al., 2007) (Figure 1A). Stimulation of LTin cells by the RET ligand artemin (ARTN) expressed on $VCAM-1^+$ stromal cells initiates upregulation of $LT\alpha 1\beta 1$ and clustering of LTin cells, resulting in activation of lymphoid tissue organizer (LTo) cells and recruitment of lymphoid tissue inducer (LTI) cells (Patel et al., 2012). In PPs, this initial event is dependent on RET and cellular adhesion but not on chemokines. It is not yet clear whether RET signaling is also involved in the development of colonic patches. LTI cells originate in the fetal liver, although recently the fetal gut has been shown to contain pre-LTI ($IL-7R\alpha^+CD4^-ROR\gamma t^-$) and LTi_0 ($IL-7R\alpha^+CD4^-ROR\gamma t^+$) cells that mature locally in response to retinoic acid (RA) signaling (van de Pavert et al., 2014). Increasing maternal retinoid intake or blocking RA signaling in

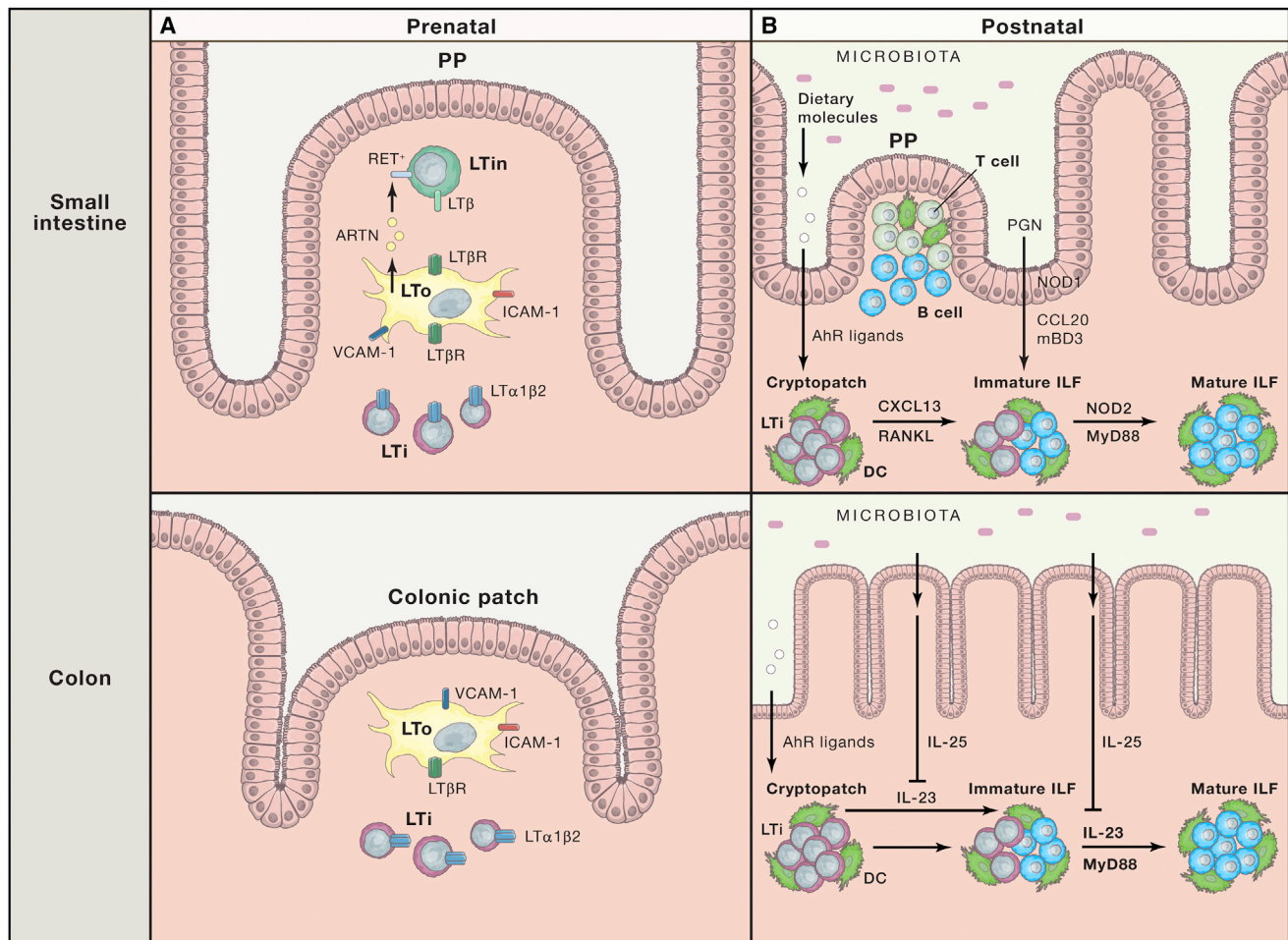


Figure 1. Pre- and Postnatal Development of GALTs

(A) PP development in the small intestine is initiated prenatally after interaction between $LT\beta^+RET^+$ LTin cells and ARTN-producing $VCAM-1^+ICAM-1^+$ LTo stromal cells. $LT\beta$ interaction with $LT\beta R$ upregulates $VCAM-1$ and $ICAM-1$ expression on LTo cells and stimulates production of cytokines CCL19, CCL21, CXCL13, and IL-7, the latter of which recruits $IL-7R\alpha^+$ LTi cells and upregulates $LT\alpha 1\beta 1$ expression. It is not known whether $LT\beta^+RET^+$ LTin cells are required for the development of colonic patches.

(B) CPs and ILFs develop postnatally in the colon and small intestine. CPs form first as clusters of LTi cells with $CD11c^+$ cells and survive and expand in response to AhR ligands. In the small intestine, the transition of CPs to immature ILFs requires RANKL and CXCL13 and microbial stimulation of NOD1 on epithelial cells, leading to the production of CCL20 and mBD3, ligands of CCR6. Further maturation involves NOD2 and the adaptor molecule MyD88. In the colon, the transition of CPs to ILFs does not require CXCL13, RANKL, CCR6, or the microbiota. Here, IL-23 production from $CD11c^+$ cells in the CPs drives maturation, which is inhibited by the IL-25 that is produced by epithelial cells in response to the microbiota.

pregnancy alters PP development, indicating that in addition to the intrinsic and programmed events that guide PP development, maternal dietary signals can also play a role (van de Pavert et al., 2014).

In PPs and colonic patches, $LT\beta R$ signaling on LTo cells leads to activation of $NF\kappa B$ (Dejardin et al., 2002) and stimulates expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), mucosal vascular addressin cell adhesion molecule 1 (MadCam-1), CXCL13, CXCL12, CCL19, CCL21, and IL-7 (Bénézech et al., 2010; Cupedo et al., 2004; Dejardin et al., 2002; Vondenhoff et al., 2009). These interactions create a positive-feedback loop leading to further recruitment of $IL-7R\alpha^+$ LTi and other hematopoietic cells, including B and T cells. $IL-7R\alpha$ signaling on LTi cells is necessary for PP development (Adachi et al., 1998; Luther et al., 2003; Yoshida et al., 1999). Whereas B and T cells are rapidly recruited

to small intestinal PPs during development, recruitment to the colonic patches is delayed (Baptista et al., 2013), suggesting differential signaling between the small intestine and the colon.

In mice, CPs and ILFs develop postnatally in a process that is influenced by dietary and microbial factors, whereas in humans, ILFs are present before birth (Hoorweg and Cupedo, 2008). In mice, CPs form around day 14 after birth and first appear as clusters of LTi cells surrounded by $CD11c^+$ cells (Figure 1B). The microbiota appears not to be required for CP formation given that such structures also form in germ-free mice (Kanamori et al., 1996). In contrast, CPs are severely reduced in the absence of the aryl hydrocarbon receptor (AhR), most likely because of a lack of expansion or survival of $ROR\gamma t^+$ innate lymphoid cells (ILCs) (Kiss et al., 2011; Lee et al., 2011). Natural AhR ligands can be endogenous, provided through diet, or produced by the microbiota through tryptophan metabolism (Cella and Colonna, 2015).

In the small intestine, the transition of CPs to ILFs requires stromal cell expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates CXCL13 production by stromal cells and CD11c⁺ DCs (Knoop et al., 2011). Deficiency of either CXCL13 or CXCR5 leads to an absence of ILFs (but not CPs) in the small intestine (McDonald et al., 2010; Velaga et al., 2009). In contrast, colonic ILFs do not require RANKL or CXCL13 for development (Baptista et al., 2013; Knoop et al., 2011). The transition of CPs to immature ILFs in the small intestine occurs in response to peptidoglycan (PGN) derived from gram-negative bacteria. PGN ligation of nucleotide-binding oligomerization domain-containing protein 1 (NOD1) on epithelial cells leads to secretion of CCL20 and β -defensin 3 (mBD3), which are both ligands for CCR6, and a deficiency of CCR6, CCL20, or mBD3 leads to loss of ILFs in the small intestine (Bouskra et al., 2008). Further maturation is then stimulated by activation of Toll-like receptors (TLRs), myeloid differentiation factor 88 (MyD88), and NOD2 (Bouskra et al., 2008). In contrast to the small intestine, the colon of germ-free mice harbors immature and mature ILFs, indicating that, despite the increased bacterial load of the colon, microbial-derived signals are not essential for their development (Baptista et al., 2013; Donaldson et al., 2015). Nevertheless, the microbiota is still able to regulate colonic ILFs. For example, the number of ILFs in the colon is increased in NOD1-deficient mice (Bouskra et al., 2008), suggesting that NOD1-independent pathways can stimulate colonic ILF development. Chemokine and cytokine involvement in ILF development also differs between the small intestine and colon; colonic ILFs are present in CCR6-deficient mice (Baptista et al., 2013), indicating that the CCR6-CCL20 pathway is less important in the colon. In addition, despite their presence in germ-free mice, mature colonic ILFs still require the adaptor molecule MyD88 (Baptista et al., 2013), suggesting that signaling through members of the IL-1R family rather than TLRs could be important at this site. Furthermore, IL-23 has been shown to be a colon-specific regulator of ILFs, which is supported by the observation that IL-23p19-deficient mice show a specific reduction in colonic ILFs (Donaldson et al., 2015). Microbial stimulation of epithelial cells leads to IL-25 production, which then inhibits IL-23 expression by CD11c⁺ cells enriched with colonic ILFs (Donaldson et al., 2015). Although microbiota stimulate IL-25 production in both the small and large intestines, tissue specificity of the response could be due to the scarcity of the IL23-expressing CD11c⁺ cells in small intestinal ILFs.

Regionalized Variation in Intestinal Adaptive Immune Cell Effector Compartments

The intestinal epithelium and underlying LP are the major sites of adaptive immune cell accumulation within the intestine. Although the overwhelming majority of adaptive immune cells in the epithelium are CD8⁺ intraepithelial lymphocytes (IELs) (Cheroute and Madakamutil, 2004), the LP contains large numbers of functionally diverse CD4⁺ T cell subsets and plasma cells (PCs) and a relatively minor fraction of CD8⁺ T cells. Collectively, these cells play an essential role in maintaining intestinal homeostasis; however, their composition differs markedly between distinct intestinal segments. Below, we highlight some of the major differences in adaptive immune cell composition between intestinal segments to emphasize the importance of local

signals in shaping regional immune specialization. Most studies have been performed in mice, and although it is tempting to extrapolate such findings to the human setting, far more work is required for assessing variations in immune cell subset composition within different segments of the human intestine under distinct physiological and pathophysiological conditions.

Variations in IEL Composition along the Length of the Intestine

IELs can be broadly divided into two major subsets: type A and type B, the proportions of which vary with age, species, and location along the length of the intestine (Figure 2). Type A IELs derive from conventional naive CD8 $\alpha\beta$ ⁺TCR $\alpha\beta$ ⁺ or CD4⁺TCR $\alpha\beta$ ⁺ T cells that have undergone activation in GALTs or LNs and subsequently homed to the intestine to generate an epithelial resident effector memory T cell population. Type b IELs, on the other hand, do not express CD8 β or CD4; instead, they express the CD8 $\alpha\alpha$ homodimer and either an $\alpha\beta$ or $\gamma\delta$ TCR. CD8 $\alpha\alpha$ ⁺TCR $\alpha\beta$ ⁺ IELs are autoreactive and appear to derive from agonist-selected double-positive thymocytes that seed the epithelium as CD4⁺CD8⁺ cells, where they upregulate CD8 $\alpha\alpha$ (Gangadharan et al., 2006; Leishman et al., 2002; Pobezinsky et al., 2012). The origin of CD8 $\alpha\alpha$ ⁺TCR $\gamma\delta$ ⁺ IELs is less clear, although recent evidence suggests that V γ 7⁺ IELs, representing the major population of TCR $\gamma\delta$ IELs in mice, undergo selective maturation and expansion within the intestine (Di Marco Barros et al., 2016). In both humans and mice, IEL numbers are greatest in the proximal small intestine and decrease down the length of the intestine. In mice, type B IELs represent a larger fraction of the IEL compartment in the small intestine than in the colon, whereas in humans, type B IELs represent only a minor fraction of IELs in each intestinal segment (Beagley et al., 1995; Boll et al., 1995; Camerini et al., 1993; Ibraghimov and Lynch, 1994; Lundqvist et al., 1995; Suzuki et al., 2002).

Germ-free mice have a marked reduction in IELs, particularly type A IELs (Bandeira et al., 1990; Kawaguchi et al., 1993; Suzuki et al., 2002), most likely as a result of reduced lymphoid tissue maturation and antigen-dependent naive T cell priming. The mechanism(s) by which the microbiota promote type B IEL homeostasis is less clear but could include bacterial-mediated NOD2-dependent IL-15 production by intestinal antigen-presenting cells (Jiang et al., 2013). Although the role of MyD88 in IEL homeostasis remains controversial (Iiyama et al., 2003; Ismail et al., 2011; Qiu et al., 2016; Yu et al., 2006), bacterial-induced MyD88 signaling in small intestinal epithelial cells appears important in maintaining type B TCR $\gamma\delta$ IEL functionality (Ismail et al., 2011).

Germ-free mice still have large numbers of type B IELs and maintain a bias for these cells in the small intestine (Suzuki et al., 2002), indicating a role for additional tissue-specific cues in regulating regionalized IEL subset composition (Figure 2). One such factor is the chemokine CCL25. CCL25 is constitutively expressed by epithelial cells in the small intestine but not the colon (Kunkel et al., 2000; Svensson et al., 2002), and its expression, which is microbiota independent, decreases from the proximal to the distal small intestine (Ericsson et al., 2004; Stenstad et al., 2007). Mice deficient in CCL25 or its receptor CCR9 have reduced numbers of small intestinal IELs and TCR $\gamma\delta$ IEL in particular (Wurbel et al., 2007; Wurbel et al., 2001). The CCL25-CCR9 axis is also required for optimal

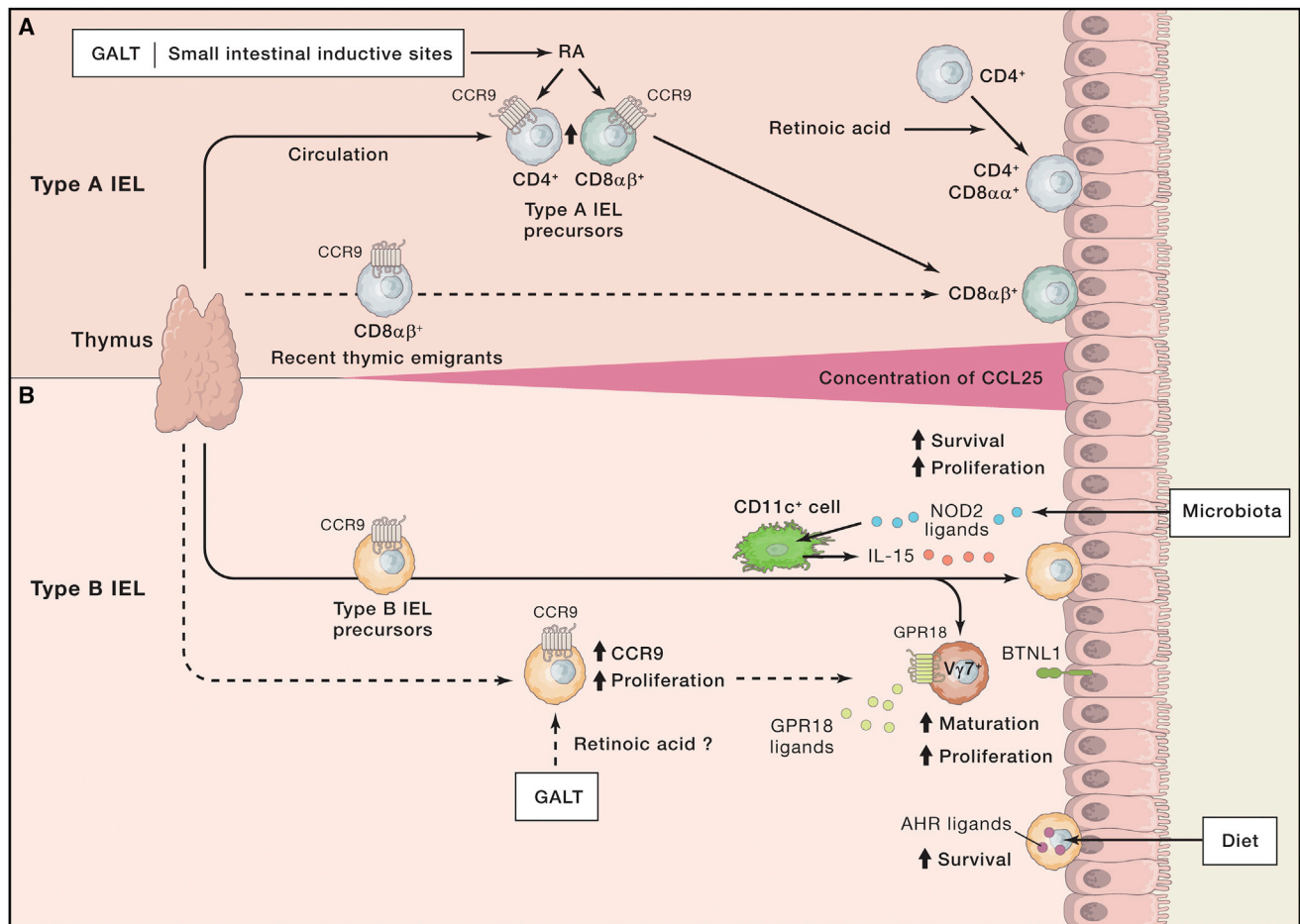


Figure 2. Factors Implicated in Regulating Intraepithelial Lymphocyte Composition along the Length of the Intestine

CCL25 is constitutively expressed by epithelial cells in the small intestine, but not the colon, particularly in the proximal small intestine. CCL25 recruits CCR9-expressing IEL precursors from the blood stream into the small intestinal epithelium.

(A) Most type A IELs derive from naive conventional CD4⁺ or CD8 $\alpha\beta$ ⁺ T cells that acquire CCR9 expression after their activation in small intestinal inductive sites and exposure to RA. CD8 $\alpha\beta$ ⁺ recent thymic emigrants also express CCR9 and can gain direct access to the small intestinal epithelium. RA is also required for the conversion of CD4⁺ type A IELs into MHCII-restricted cytotoxic CD4⁺CD8 $\alpha\alpha$ ⁺ IELs.

(B) CCR9-expressing type B IEL precursors are recruited directly from the thymus, although some might undergo prior circulation and activation in small intestinal GALTs and acquire higher expression of CCR9. After their entry into the small intestinal epithelium, type B IEL numbers are maintained by multiple local factors, including (1) microbial-derived NOD ligands that drive resident antigen-presenting cells to produce IL-15, a proliferation and survival factor for type B IEL; (2) selective expression of BTNL1 by small intestinal epithelial cells to promote V γ 7⁺ IEL maturation and proliferation; (3) GPR18 ligands that promote type B IELs, particularly V γ 7⁺ IEL maturation; and (4) AhR ligands that directly promote type B IEL survival.

CD8 $\alpha\beta$ ⁺ T cell migration to the small intestinal epithelium (Johansson-Lindbom et al., 2003; Svensson et al., 2002; Wurbel et al., 2007), especially the proximal small intestine (Stenstad et al., 2007). CCR9 is induced on CD8 $\alpha\beta$ ⁺ T cells during their activation in intestinal inductive sites (Johansson-Lindbom et al., 2003; Svensson et al., 2002), a process that is dependent on the vitamin A metabolite RA (Iwata et al., 2004; Jaensson-Gyllenbäck et al., 2011; Svensson et al., 2008). Vitamin A obtained through diet is found at higher concentrations in the small intestine than in the colon (Jaensson-Gyllenbäck et al., 2011) and is locally converted to RA by small intestinal epithelial cells (McDonald et al., 2012) and local stromal cells (Vicente-Suarez et al., 2015). DCs in the small intestine constitutively receive stronger RA signals than do colon DCs (Jaensson-Gyllenbäck et al., 2011), imprinting them with an ability to generate RA and, after their migration to intestinal draining LNs, induce

CCR9 on responding T cells. Some type B IEL precursors, particularly V γ 7⁺ T cells, can also proliferate and acquire enhanced CCR9 expression in small intestinal GALTs prior to their entry into the small intestinal epithelium (Guy-Grand et al., 2013). RA is also required for the differentiation of CD4⁺ type A IELs into major histocompatibility complex II (MHCII)-restricted cytotoxic CD4⁺CD8 $\alpha\alpha$ ⁺ IELs (Reis et al., 2014). Indeed, given the pleiotropic effects of RA on immune cells, its broader impact on small intestinal IEL functionality warrants further study.

The butyrophilin-like (btln) molecules are an additional family of mediators involved in regulating region-specific lymphocyte composition in epithelial tissues (Abeler-Dörner et al., 2012). Murine BTNL1, BTNL4, and BTNL6 are selectively expressed in the small intestine by post-mitotic enterocytes; BTNL1 is predominantly present in the proximal and middle segments of the small intestine and reaches maximal expression 14 days

after birth (Bas et al., 2011; Di Marco Barros et al., 2016). Enterocyte-expressed BTNL1 is required for the maturation and proliferation of $V\gamma 7^+$ IELs after their entry into the epithelium, a process that is independent of the thymus, the microbiota, and dietary proteins (Di Marco Barros et al., 2016). Induced expression of a *Btnl1* transgene in young (7- or 21-day-old) but not adult (11-week-old) *Btnl1*-deficient mice rescues $V\gamma 7^+$ IEL proliferation and numbers, suggesting that BTNL1-mediated $V\gamma 7^+$ IEL proliferation is temporally restricted to early life. G-protein-coupled receptor 18 (*Gpr18*)-deficient mice also have reduced numbers of small intestinal IELs, particularly $V\gamma 7^+$ IELs (Wang et al., 2014). Similar to BTNL1, GPR18 appears to function after $\gamma\delta^+$ T cell entry into the epithelium, and $\gamma\delta^+$ IELs in *Gpr18*-deficient mice appear immature (Wang et al., 2014). Although the identity of the GPR18 ligand(s) remains unclear, these results indicate that GPR18 could function to promote $V\gamma 7^+$ IEL interactions with BTNL1-expressing enterocytes. Interestingly, although the genetic locus encompassing *Btnl1* and *Btnl6* is lacking in humans, BTNL3 and BTNL8 are constitutively expressed by epithelial cells in the human colon, and in vitro studies indicate their potential role in supporting human $V\gamma 4^+$ IEL maturation (Di Marco Barros et al., 2016).

Finally, a major dietary determinant of type B IEL homeostasis is dietary AhR ligands (Li et al., 2011). Mice deficient in AhR have a selective reduction in type B IELs in the small intestine. Wild-type mice fed a synthetic diet have reduced numbers of type B IELs, but these can be restored by dietary supplementation with the AhR ligand precursor indole-3-carbinol (I3C). Mechanistically, direct AhR signaling in IELs appears important for type B IEL survival after their entry into the epithelium. Whether AhR signaling contributes to type B IEL homeostasis in other parts of the intestine remains to be explored.

Regional Compartmentalization of LP $CD4^+$ T Cell Subsets

The selective induction of homing molecules in intestinal inductive sites is a major contributor to the generation of regionalized LP T cell compartments. CCR9 induction on $CD4^+$ and $CD8^+$ T cells after activation in small intestinal inductive sites promotes their homing to the small intestinal LP, particularly to the proximal small intestine (Campbell and Butcher, 2002; Stenstad et al., 2006; Stenstad et al., 2007; Svensson et al., 2002). Conversely, the G-protein-coupled receptor GPR15 is expressed on colonic but not small intestinal T cells, and in mice, it promotes effector and regulatory T cell recruitment to the colonic LP (Kim et al., 2013; Nguyen et al., 2015). In contrast to CCR9, where and how activated T cells acquire expression of GPR15 remains unclear, although it is attractive to speculate that this could occur in colonic GALT or colon draining LNs. Administration of broad-spectrum antibiotics in mice reduces GPR15 expression on colonic T cells (Kim et al., 2013), and SCFAs induce expression of GPR15 on anti-CD3-treated human $CD4^+$ T cells in vitro (Fischer et al., 2016), suggesting a potential role for the microbiota in GPR15 induction. The GPR15 ligand(s) mediating T cell recruitment to the colon are unknown, although GPR15-dependent T cell homing to the colon is unaffected in germ-free mice, indicating that the ligands are not microbial derived (Kim et al., 2013). In contrast to murine GPR15, the human protein is expressed on intestinal effector cells but not Foxp3⁺ Treg cells,

suggesting that GPR15 is a potential target for the treatment of colitis (Fischer et al., 2016; Nguyen et al., 2015).

Although distinct homing pathways help focus effector and regulatory T cell responses to the small intestine or colon, there are also marked differences in the composition of LP T helper (Th) subsets, particularly in the proportions of Foxp3⁺ Treg and Th17 cells along the length of the mouse intestine. The proportions of Foxp3⁺ Treg cells in the intestinal LP are higher than in other tissues; they range from 10%–20% of $CD4^+$ T cells in the small intestine and 20%–40% in the colon, depending on housing conditions. Strikingly, Foxp3⁺ Treg cell numbers are reduced in the colon but not small intestine of germ-free or antibiotic-treated mice (Atarashi et al., 2011; Geuking et al., 2011), whereas germ-free mice fed an antigen-free diet have dramatically reduced numbers of small intestinal Foxp3⁺ Treg cells (Kim et al., 2016a). Thus, most Foxp3⁺ Treg cells in the small intestine appear to develop in response to dietary antigens, whereas a large proportion of colonic Foxp3⁺ Treg cells develop in response to the microbiota. During homeostasis, LP Foxp3⁺ Treg cells can be divided into two major subsets: (1) Foxp3⁺ Treg cells that co-express neuropilin 1 (NRP1) and Helios and are believed to primarily consist of thymically derived natural (n)Treg cells and (2) Helios[−]NRP1[−]Foxp3⁺ Treg cells that appear to represent peripherally induced Treg (pTreg) cells. pTreg cell subsets can be further divided into ROR γ t[−]-expressing Foxp3⁺pTreg cells and ROR γ t[−]Foxp3⁺ pTreg cells (Ohnmacht et al., 2015; Sefik et al., 2015; Yang et al., 2016). ROR γ t[−]Foxp3⁺ pTreg cells represent the major Foxp3⁺ Treg cell subset in the colon and, in contrast to natural Treg (nTreg) or ROR γ t[−]Foxp3⁺ pTreg cells, are dramatically reduced in germ-free mice or after antibiotic treatment, suggesting that they develop in response to the microbiota (Kim et al., 2016a; Ohnmacht et al., 2015; Sefik et al., 2015; Yang et al., 2016). Consistent with this, mono-colonization of germ-free mice with bacteria from a range of phyla and genera induces colonic ROR γ t[−]Foxp3⁺ pTreg cell generation, albeit to varying degrees (Sefik et al., 2015); a dominant species possessing such activity in both humans and mice appears to be the Clostridia that promote mucosal TGF β production and ROR γ t[−]Foxp3⁺ pTreg cell generation independently of MyD88 (Atarashi et al., 2013; Atarashi et al., 2011; Ohnmacht et al., 2015). Interesting, ROR γ t[−]Foxp3⁺ Treg cell numbers are also reduced in IL-6- and IL23 α -deficient mice, suggesting that they follow a developmental pathway at least partially overlapping that of Th17 cells (Ohnmacht et al., 2015). The microbiota has also been suggested to maintain the intestinal nTreg cell niche under conditions of pTreg cell deficiency in an MHCII-independent manner (Korn et al., 2014). In contrast, ROR γ t[−]Foxp3⁺ pTreg cells dominate in the small intestine and represent only a minor proportion of Foxp3⁺Treg cells in the colon, and their numbers are selectively reduced in mice fed an antigen-free diet, suggesting that they develop in response to dietary antigen (Kim et al., 2016a). Global absence of Foxp3⁺pTreg cells in the intestine results in spontaneous allergic Th2-type inflammation along the length of the intestine (Josefowicz et al., 2012); however, ROR γ t[−] and ROR γ t[−] pTreg cell subsets appear to play distinct roles in maintaining homeostasis given that the specific absence of ROR γ t[−]Foxp3⁺ pTreg cells results in enhanced intestinal Th2 (Ohnmacht et al., 2015) or Th17 and Th1 cell responses (Sefik et al., 2015), whereas the absence of

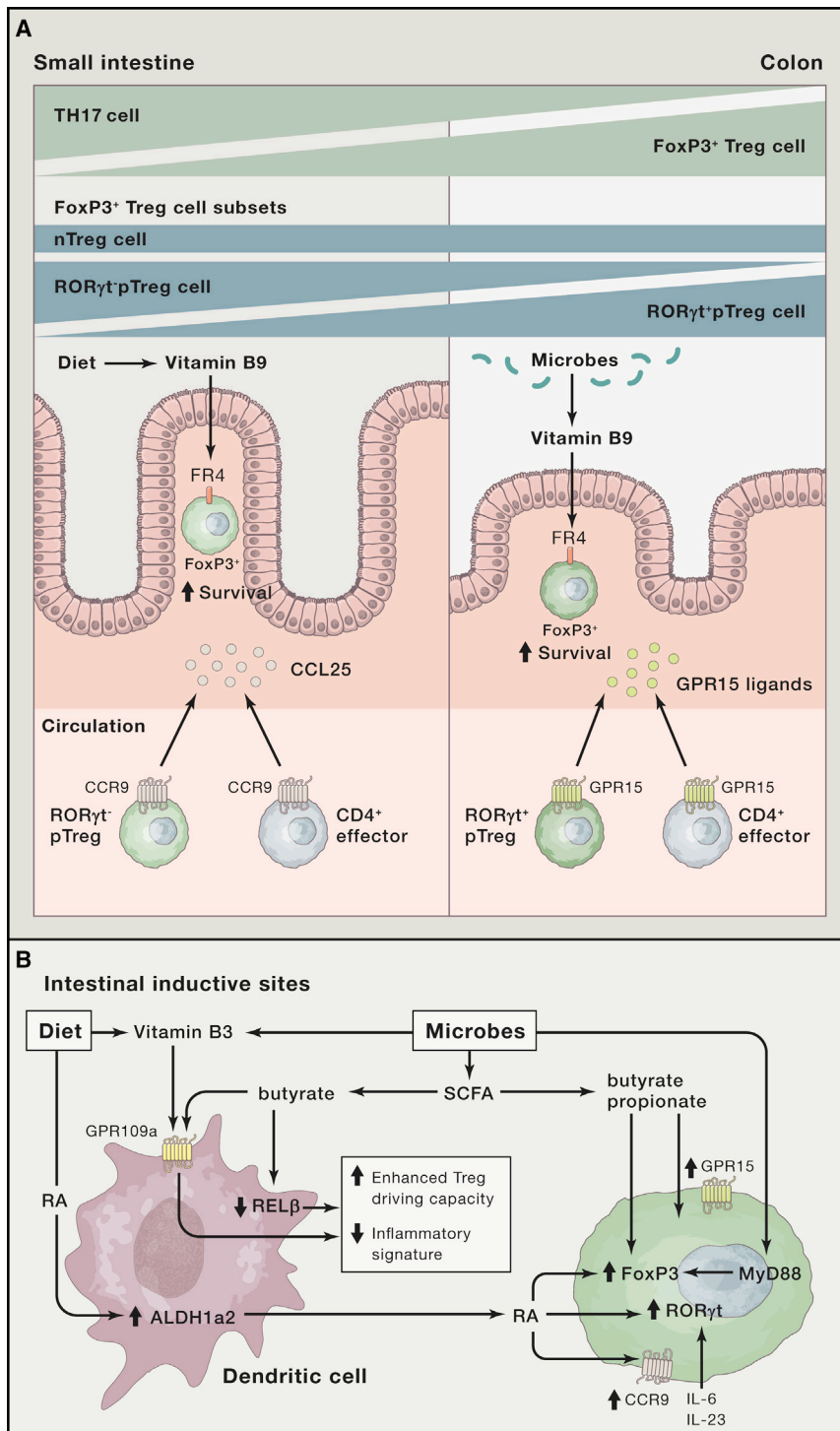


Figure 3. Impact of Environmental Signals on the Generation and Distribution of Peripherally Induced FoxP3⁺ Treg Cells

(A) FoxP3⁺ Treg cells, although found throughout the intestinal LP, are highest in number in the colon and inversely correlate with intestinal Th17 cell numbers. FoxP3⁺ Treg cells can be divided into Helios⁺NRP1⁺ thymically derived nTreg cells and Helios⁺NRP1⁺ pTreg cells. pTreg cells can be further divided into RORγt⁺pTreg cells, which are heavily dependent on the microbiota and dominate in the colon, and RORγt⁺ pTreg cells, which are dependent on dietary antigen and dominate in the small intestine. Dietary or microbial-derived vitamin B9 signals through folate receptor 4 (FR4) to promote FoxP3⁺ Treg cell survival in the intestinal LP. FoxP3⁺ Treg cell and effector CD4⁺ T cell migration to the small intestine involves the CCL25-CCR9 axis, whereas FoxP3⁺ Treg cell (mouse only) and effector CD4⁺ T cell migration to the colon is mediated by GPR15, whose ligands remain to be determined.

(B) pTreg cells are generated from naive CD4⁺ T cells in intestinal inductive sites, and their development requires MHCII expression by CD11c⁺ DCs. The generation of pTreg cells is enhanced by environmental signals acting directly on CD4⁺ T cells during their differentiation or indirectly on DCs promoting their tolerogenic capacity. For RORγt⁺ pTreg cells, such signals include microbial-dependent MyD88 signaling and SCFA signaling in T cells to promote FoxP3 transcription and potentially expression of the colon homing receptor GPR15. Generation or maintenance of RORγt⁺pTreg cells also requires IL-6 and IL-23, which promote RORγt expression, and RA, which promotes FoxP3 expression and could prevent these cells from differentiating to the Th17 cell lineage. The ability of DCs to drive FoxP3⁺ Treg cell differentiation is promoted by RA, which in a feed forward loop enhances the capacity of DCs to make RA and vitamin B3 (niacin), which functions via GPR109a, to reduce the inflammatory potential and promote the regulatory potential of these cells. Butyrate signals through GPR109a in a similar manner or in a G-protein-coupled-independent manner to downregulate RelB expression and enhance the Treg-cell-driving capacity of DCs. The generation of RORγt⁺ pTreg cells is independent of the microbiota, IL-6, and IL-23 but is promoted by RA. RA synergizes with TGFβ to drive RORγt⁺ pTreg cell development and simultaneously induces expression of CCR9, priming these cells with a small intestinal homing capacity.

signaling in FoxP3⁺ Treg cells is important for optimal pTreg cell induction potentially via demethylation of conserved non-coding sequence 2 (CNS2) of the *Foxp3* promoter, which is required for stable *Foxp3* expression. As a result, mice with MYD88 deficiency specifically in FoxP3⁺

RORγt⁺FoxP3⁺ pTreg cells enhances susceptibility to food antigen (Kim et al., 2016a).

In addition to providing a steady source of antigen, diet and the microbiota support intestinal Treg cell homeostasis by a wide variety of mechanisms, many of which remain incompletely understood (for a recent review, see Tanoue et al., 2016) (Figure 3). Direct microbiota-induced MyD88-dependent

Treg cells have reduced numbers of intestinal FoxP3⁺ Treg cells (Wang et al., 2015). Short-chain fatty acids (SCFAs), bacterial metabolites generated from the fermentation of undigested fiber and present in high levels in the colon (Cummings et al., 1987), also promote colonic FoxP3⁺Treg cell homeostasis. Butyrate acts directly on CD4⁺ T cells to drive H3 acetylation of the *FoxP3* promoter and its CNS1 enhancer element, required for

intestinal pTreg cell generation (Arpaia et al., 2013; Furusawa et al., 2013), whereas propionate enhances pTreg cell generation (Arpaia et al., 2013) and colonic nTreg cell proliferation and functionality, the latter via GPR43 (Smith et al., 2013). Butyrate also promotes FoxP3⁺ Treg cell generation indirectly through DCs, either in a G-protein-coupled-receptor-independent manner to suppress RelB and pro-inflammatory gene expression (Arpaia et al., 2013) or via the butyrate and niacin (vitamin B3) receptor GPR109a (Singh et al., 2014), enhancing their ability to drive de novo FoxP3⁺ Treg cell differentiation. Consistent with the latter, mice deficient in *Niacr1* (which encodes GPR109a) display a marked reduction of colonic but not small intestinal FoxP3⁺ Treg cells (Singh et al., 2014). Certain commensals have also adapted unique mechanisms to promote intestinal Treg cell homeostasis and host-microbial mutualism. Perhaps the best characterized mechanistically is polysaccharide A (PSA) from the human commensal *Bacteroides fragilis*; it promotes IL-10 production by intestinal FoxP3⁺ Treg cells via TLR2-dependent direct effects on CD4⁺ T cells (Round and Mazmanian, 2010) or via indirect effects on plasmacytoid DCs (Dasgupta et al., 2014) or conventional DCs (Shen et al., 2012).

Several dietary metabolites that promote FoxP3⁺ Treg cell generation have also been identified. In addition to inducing CCR9 on pTreg cells, RA synergizes in vitro with TGFβ to promote pTreg cell differentiation (Kang et al., 2007; Sun et al., 2007), and consistent with this finding, mice kept on a vitamin-A-deficient diet appear defective in the induction of oral tolerance (Cassani et al., 2011). RA also promotes the generation of RORγt⁺FoxP3⁺ pTreg cells in vitro (Lochner et al., 2008), and blocking RA signaling in vivo prevents the development of these cells (Ohnmacht et al., 2015). Nevertheless, RA has pleiotropic roles in regulating immune cell functionality (for a review see, Brown and Noelle, 2015), and the direct role of RA signaling in T cells for intestinal nTreg and pTreg cell homeostasis in vivo remains to be determined. The water-soluble vitamin B subtype vitamin B9 (folic acid [FA]) also affects FoxP3⁺ Treg cell homeostasis. FA is obtained exclusively from diet or through the microbiota, and mice kept on an FA-deficient diet display a selective reduction in intestinal FoxP3⁺ Treg cells (Kinoshita et al., 2012; Kunisawa et al., 2012). Local concentrations of FA promote FoxP3⁺ Treg cell survival through folate receptor 4, which is highly expressed by FoxP3⁺ Treg cells (Kinoshita et al., 2012; Yamaguchi et al., 2007). Although additional dietary derivatives have been implicated in promoting Treg cell induction, most notably tryptophan metabolites (Mezrich et al., 2010; Singh et al., 2014) and the vitamin D3 metabolite 1-25 dihydroxyvitamin D3 (Kang et al., 2012), their impact on regionalized intestinal FoxP3⁺ Treg cell homeostasis remains to be elucidated.

In mice, the total number and proportions of intestinal Th17 cells along the length of the intestine inversely correlate with those of FoxP3⁺ Treg cells, such that the highest Th17 cell numbers are found in the proximal small intestine and steadily decline toward the colon (Denning et al., 2011). Th17 cells are virtually absent from the intestine of germ-free mice but are found in normal numbers in *Myd88*^{-/-}*Trif*^{-/-} mice, demonstrating a key role for the microbiota, but not TLR signaling, in intestinal Th17 cell homeostasis (Atarashi et al., 2008; Ivanov et al., 2008). Treatment of mice with broad-spectrum antibiotics results in a severe depletion of intestinal Th17 cells, suggesting that the microbiota

is also critical for maintenance of the intestinal Th17 cell compartment (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2008). The best characterized microbial inducer of intestinal Th17 cell responses is the gram-positive segmented filamentous bacteria (SFB) (Ivanov et al., 2009). SFB adhere to epithelial cells in the terminal ileum and drive a dramatic increase in Th17 cell numbers within the ileal LP. Mechanistically, SFB induce an antigen-specific CD4⁺ T cell response that is heavily skewed toward the RORγt⁺ Th17 cell lineage (Geem et al., 2014; Goto et al., 2014; Yang et al., 2014) and dependent on MHCII expression by CD11c⁺ cells (Geem et al., 2014; Goto et al., 2014) and monocyte-derived macrophages (Panea et al., 2015). SFB-specific Th17 cell differentiation can occur independently of GALT and MLN (Geem et al., 2014; Lécuyer et al., 2014) but most likely requires the tertiary lymphoid tissues that develop as a result of SFB colonization (Lécuyer et al., 2014). Recent data suggest that newly generated SFB-specific RORγt⁺ CD4⁺ T cells disseminate throughout the gut (Sano et al., 2015) and that epithelial-derived serum amyloid A (SAA) and reactive oxygen species (ROS) promote IL-17 production by infiltrating RORγt⁺CD4⁺ T cells within the ileum (Atarashi et al., 2015; Sano et al., 2015). SFB adherence to ileal epithelial cells is required for epithelial SAA and ROS production, and amplification of this epithelial SAA response through IL-23-dependent IL-22 production by ILC3 and IL-1β production from LP CD11c⁺ cells is further required to drive local IL-17 production by infiltrating RORγt⁺CD4⁺ T cells (Atarashi et al., 2015; Sano et al., 2015). The ability to induce Th17 responses is not, however, unique to SFB but could be a general property of adherent commensal or pathogenic microbes (Atarashi et al., 2015; Tan et al., 2016b), whereby the site of intestinal IL-17⁺ CD4⁺ T cell accumulation is determined by the location of the adherent microbe. In this regard, it is interesting to speculate that easier access of adherent microbes to the small intestinal epithelium underlies the enhanced numbers of Th17 cells within this region of the intestine. Finally, although direct epithelial adherence appears key to the efficient generation of Th17 responses, the downstream molecular mechanisms driving this response are likely to be microbe specific (Atarashi et al., 2015; Tan et al., 2016b).

Given the key role of the microbiota in intestinal Th17 cell homeostasis, it is not surprising that dietary-induced changes in microbial composition can alter intestinal Th17 cell numbers. For example, alterations in microbial composition induced by a high-fat diet drive changes in small intestinal antigen-presenting cell functionality, resulting in reduced small intestinal Th17 cell numbers (Garidou et al., 2015), whereas dietary supplementation with long-chain fatty acids (LCFAs) alters the microbial composition by increasing levels of medium-chain fatty acids and LCFAs and reducing SCFAs to promote small intestinal Th17 development (Haghikia et al., 2015). Thus, bacterial communities generating high concentrations of SCFAs promote intestinal Treg cell development, whereas those generating LCFAs appear to favor Th17 cell development. Finally, luminal ATP also appears to promote intestinal Th17 development (Atarashi et al., 2008; Kusu et al., 2013).

Regional Compartmentalization of IgA⁺ PCs

An estimated 80% of human and mouse PCs are located in the intestinal LP, and recent evidence suggests that they can be

extremely long lived (Landsverk et al., 2017). Their density is highest in the proximal and distal portions of the intestine, and the overwhelming majority of these produce IgA (approximately 75%–80% of PCs in duodenum and jejunum and 90% of PCs in the colon). In both mice and humans, intestinal IgA⁺ PCs represent a collection of low-frequency and expanded clones (Lindner et al., 2012) that display specificity for the microbiota and self-antigens (Benckert et al., 2011). Intestinal IgA plays an essential role in establishing luminal microbial diversity and host-microbiota mutualism and contributes to preventing microbial access to systemic compartments (Fagarasan et al., 2002). In contrast to mice, which have one IgA subclass, humans have IgA1 and IgA2; IgA1-producing PCs dominate in the small intestine, and IgA2 PCs dominate in the colon (Crango et al., 1984; Kett et al., 1986; Lin et al., 2014). Because protein antigens tend to drive IgA1 responses and polysaccharides tend to drive the IgA2 subclass (Tarkowski et al., 1990), the differential distribution of these subclasses along the length of the intestine could reflect alterations in luminal content. Consistent with this, the dominance of IgA1 in the small intestine is reversed to IgA2 under conditions of bacterial outgrowth (Kett et al., 1995). IgA2 is also more resistant to bacterial proteases, suggesting adaptation to the colonic environment (Kilian et al., 1996; Plaut et al., 1974).

GALTs are considered the major sites of IgA class switching (Barone et al., 2011; Brandtzaeg, 2009), which (in mice) optimally requires M-cell-mediated uptake of luminal antigen (Rios et al., 2016). Whereas in mice large GALTs have been considered the primary site of T-cell-dependent IgA responses and ILFs the primary site of T-cell-independent IgA responses (Tsuji et al., 2008), it remains unclear whether this division of labor occurs in humans.

Several host-derived factors promote class switching to IgA in GALTs in vivo; the principal factor in mice is TGF β (Cazac and Roes, 2000; van Ginkel et al., 1999). However, induction of IgA class switching and PC generation is also highly dependent on external diet- and microbial-derived signals (Figure 4). Germ-free mice display a dramatic reduction in intestinal IgA PCs (Crabbé et al., 1968). The mechanisms by which the microbiota promotes IgA class switching and IgA PC generation in GALTs are multifaceted and remain to be fully characterized. In addition to providing relevant antigenic material and promoting GALT development, the microbiota directly signals through MyD88 in T cells to drive germinal center (GC) T follicular helper (T_{FH}) cell generation in PPs and subsequent T-cell-dependent secretory IgA responses to the microbiota (Kubinak et al., 2015). Microbial-driven MyD88-dependent iNOS induction in CD11c⁺ cells also promotes T-cell-dependent IgA switching through the induction of TGF β RII on B cells and T-cell-independent IgA responses through induction of a proliferation-inducing ligand (APRIL) and B-cell-activating factor of the TNF family (BAFF) (Tezuka et al., 2007). Additionally, SCFAs inhibit histone deacetylase (HDAC) in B cells to enhance glycolysis and fatty acid synthesis and support antibody production, and they appear to regulate the numbers of IgA⁺ GC B cells in PPs and IgA⁺ PCs in the small intestine and colon (Kim et al., 2016b). Finally, RA promotes IgA class switching and IgA production in vitro (Mora et al., 2006; Watanabe et al., 2010), and mice whose B cells are unresponsive to RA display a marked reduction in IgA⁺ GC B cells in PPs and IgA⁺ PCs in the small intestine (Pantazi et al., 2015). Notably, the SCFA acetate

signals through GPR43 in DCs to enhance their ability to generate RA and promote IgA class-switch recombination and IgA production in vitro (Wu et al., 2016). Whether RA displays a more dominant role in driving IgA plasmablast generation in the small intestine than in the colon, as might be expected given the higher concentrations of retinol in the small intestine (Jaensson-Gyllenbäck et al., 2011), remains to be determined.

IgA plasma cell clones are not evenly distributed throughout the intestine; expanded clones detected throughout the small intestine are infrequently found in the colon of the same mouse and vice versa (Lindner et al., 2012). Such site-specific accumulation of IgA PCs within distinct intestinal segments, as with T cells, is likely to be regulated by differential expression of homing molecules (Figure 4). Consistent with this, RA-dependent CCR9 induction on IgA plasmablasts is required for optimal IgA PC accumulation in the small intestine, but not the colon (Mora et al., 2006; Pabst et al., 2004), and Ccr9 deficiency results in an enhanced overlap of related expanded IgA clones between the small intestine and colon (Lindner et al., 2012). IgA PCs also express CCR10, whose ligand CCL28 is expressed by intestinal epithelial cells, particularly in the colon. CCR10 and CCL28 are required for optimal T-cell-dependent IgA plasmablast accumulation in both the small intestine and colon (Hieshima et al., 2004; Hu et al., 2011), as well as for the maintenance of long-lived intestinal IgA⁺ PCs and IgA⁺ memory B cells (Hu et al., 2011). Although the mechanisms involved in CCR10 induction remain to be determined, newly generated plasmablasts in caecal patches express CCR9 and CCR10, whereas those generated in PPs only express CCR9 (Masahata et al., 2014). As a result, newly generated IgA⁺ plasmablasts in the caecal patch localize to both the small intestine and colon, whereas those generated in PPs migrate selectively to the small intestine (Masahata et al., 2014).

The titers of luminal IgA and the proportions of IgA-coated luminal bacteria in both humans and mice are far higher in the small intestine than in the colon (Bunker et al., 2015) (Figure 4). Under homeostasis, similar bacterial taxa are represented within IgA-bound and -unbound bacterial fractions in the small intestine, indicating that most small intestinal commensals can elicit an IgA response. In contrast, bacterial species that are selectively present in the colon have little IgA coating, suggesting that commensals in the colon for the most part do not elicit an IgA response. Interestingly, specific IgA coating of bacteria is preserved even when titers of unbound luminal IgA are dramatically reduced in the absence of T cells, suggesting that small intestinal commensals can efficiently induce T-cell-independent specific IgA responses and that most unbound IgA is dependent on T cells and specific to non-microbial antigens (Bunker et al., 2015). Nevertheless, there is clear evidence that certain commensals induce T-cell-dependent IgA (Palm et al., 2014) that can participate in bacterial coating and promote microbial diversity (for a review, see Pabst et al., 2016). Why certain commensals are capable of promoting T-cell-dependent IgA is currently unclear, although an ability to interact with the mucosal surface could be one contributing factor.

Development and Maturation of Adaptive Immune Cells in the Intestine

Postnatal development of adaptive immune cells occurs rapidly after birth. Very few $\alpha\beta$ TCR⁺ T cells are present in the mouse intestine

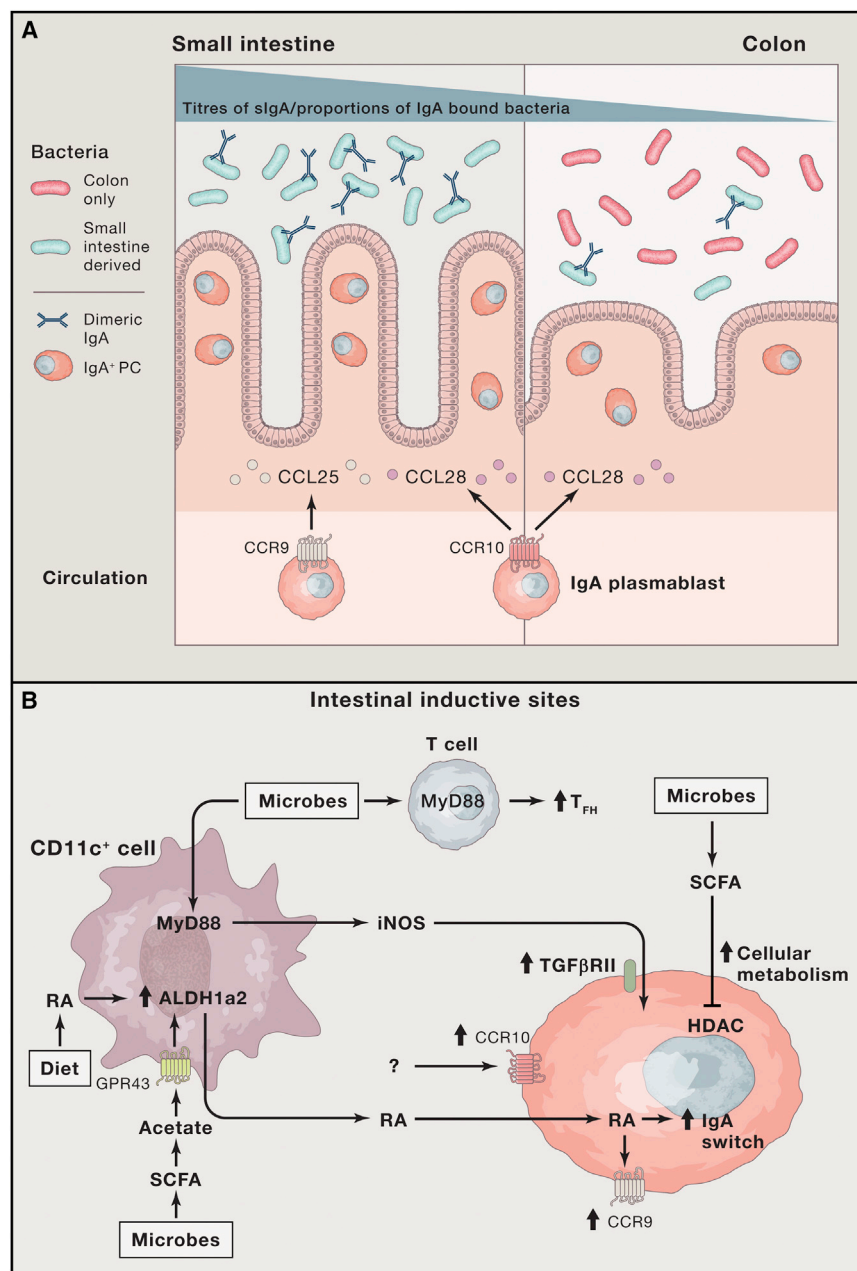


Figure 4. Impact of Environmental Signals on the Regionalized Generation and Distribution of IgA PCs

(A) Luminal concentrations of sIgA and the proportions of IgA-bound bacteria are higher in the small intestine than in the colon. Bacteria that are selectively present in the colon show little IgA coating, suggesting that most commensal-specific IgA responses take place in the small intestine. IgA plasmablast recruitment to the small intestine is mediated by CCR9 and CCR10 and that to the colon is mediated by CCR10. The CCR9-CCL25 axis appears to play a dominant role in IgA⁺ plasmablast accumulation to the small intestine and most likely underlies the largely non-overlapping distribution of dominant IgA plasma cell clones between the small intestine and colon. (B) Under homeostasis, B cells activated in intestinal inductive sites preferentially differentiate into IgA plasmablasts. TGFβ signaling in B cells is essential for IgA class switching in vivo. Bacterial-dependent MyD88 signaling in CD11c⁺ cells induces iNOS to promote TGFβRII expression on B cells. Bacterial-dependent MyD88 signaling in T cells promotes T_{FH} generation in GALTs, enhancing T-cell-dependent IgA responses. SCFAs act directly on B cells to enhance their cellular metabolism and support B cell differentiation and antibody production. GALT DCs are imprinted with the ability to generate RA via direct RA signaling and potentially through acetate-induced GPR43-dependent signals. RA from DCs, and potentially other cells, acts directly on B cells to promote IgA class switching and induce CCR9 expression on newly generated plasmablasts. IgA⁺ plasmablasts generated in PPs are induced to express CCR9, whereas those generated in the caecal patch appear to express both CCR10 and CCR9. As a result, PP-derived plasmablasts localize to the small intestine, whereas caecal-patch-derived plasmablasts can gain access to both the small intestinal and colon. The mechanisms underlying CCR10 induction on IgA⁺ plasmablasts remain unclear.

before birth, but they increase massively within 2 days (Torow et al., 2015b). The majority of the T cells that appear in the early phase are thymic-derived CD4⁺αβTCR⁺ T cells that home to early PPs, whereas CD4⁺ T cell recruitment to the LP takes place between days 11 and 28. Another wave of T cells arrives after weaning and is mostly composed of CD8αβ⁺TCRαβ⁺ T cells, although there is some contribution from CD8αα⁺TCRαβ⁺ and CD4⁺CD8αα⁺TCRαβ⁺ T cells. The very early influx of CD4⁺ T cells to the neonatal gut is independent of microbial and TLR signals but requires β7-integrin-dependent pathways. Despite ongoing microbial colonization, these early CD4⁺αβTCR⁺ T cells retain a naive phenotype through a pathway involving both maternal sIgA and Treg cells (Torow et al., 2015a; Torow et al., 2015b).

(Wesemann et al., 2013). The colon also harbors small numbers of these cells, but they are absent from PPs. These cells could help to shape the pre-immune repertoire, potentially to negatively (or positively) select against bacterial reactivity, and the dramatic decrease in their presence in the LP after weaning would suggest a limited window for this to occur. It is unclear whether an equivalent population exists in the neonatal human gut.

Microbial colonization of the intestine stimulates B cell class-switch recombination, primarily to IgA, in the PPs and ILFs. In mice, maturation of the mucosal IgA response is inhibited by the presence of maternal sIgA, and IgA⁺ PCs do not appear in the LP until after weaning (Harris et al., 2006). Maternal

anti-commensal IgG (and IgA) in the milk also dampens development of a mucosal T_{FH} cell response in neonatal mice, which limits GC B cell responses in early life (Koch et al., 2016). In humans, despite the presence of ILFs at birth and a rapid influx of B cells after birth, the development of a mucosal IgA response is also delayed until after 1 month, and this delay correlates with reduced expression of APRIL and its receptors TACI and BCMA, but not AID, in the GALTs (Gustafson et al., 2014).

Relevance for Disease Susceptibility Later in Life

Hippocrates once stated that “all disease begins in the gut.” Indeed, the intestinal microbiota contributes to host immunity, and proper development of the immune landscape within the intestine is of critical importance to decreasing susceptibility to diseases that affect both the gastrointestinal tract and systemic sites. As discussed above, the immune landscape within the intestine starts to take shape during fetal life but reaches full maturity only after birth with guidance by exogenous signals from the microbiota and diet. This dynamic phase in early life represents a critical window whereby proper or improper immune development can affect resistance or susceptibility, respectively, to diseases later in life. The development of intestinal lymphoid tissues and innate and adaptive immunity early in life defines this neonatal window.

The impact of improper immune development during this critical window and the elucidation of underlying mechanisms have best been illustrated in mouse models together with the use of gnotobiotics. Microbial colonization of adult germ-free mice does not lead to the same level of immune maturation as colonization of neonates, suggesting that exposure to the microbiota must occur within a neonatal window in order to properly imprint host immunity and homeostasis (El Aidy et al., 2013). However, germ-free mice do not just have an immature immune system—they also display signs of immune dysregulation. Germ-free mice harbor increased numbers of iNKT cells in both the lung and colon, leading to increased susceptibility to inflammation in both tissues (Olszak et al., 2012). Importantly, colonization before weaning is necessary for inhibiting the accumulation of these cells and protecting from inflammatory disease later in life, illustrating the need for microbial education during this critical window (Olszak et al., 2012). Germ-free mice also display increased serum IgE levels and increased susceptibility to anaphylaxis (Cahenzli et al., 2013). Neonatal colonization with a relatively diverse microbiota suppresses IgE induction and protects from allergy (Cahenzli et al., 2013). The requirement for a diverse microbiota suggests either that multiple microbes are necessary for the provision of a full range of immune educational cues or that a keystone microbial species normally present in a diverse microbial community is missing. Further evidence for a critical window is illustrated by the increased susceptibility to a variety of diseases in mice that received antibiotics in early life. Administration of clinical doses of select antibiotics to neonatal, but not adult, mice led to enhanced susceptibility to experimental allergic asthma, which was linked to a reduction in the number of colonic Treg cells and an increase in IgE (Russell et al., 2012; Russell et al., 2013). In another study, antibiotic treatment during early life led to an increased sensitivity to food allergy via depletion of beneficial *Clostridia* spp., and

supplementation stimulated IL-22 production, leading to an enhanced epithelial barrier and reduced bacterial translocation (Stefka et al., 2014).

The impact of antibiotic use is not limited to Th2-cell-mediated immunity. In the non-obese diabetic (NOD) mouse model of type 1 diabetes (T1D), pulses of antibiotics administered in early life altered the composition and metabolism of the gut microbiome and led to changes in T cell populations and gene expression in the small intestine, which accelerated the onset of T1D (Livanos et al., 2016). Antibiotic treatment during pregnancy can also alter the development of T1D in the offspring by changing the intestinal immune landscape (Hu et al., 2015; Tormo-Badia et al., 2014), such that different antibiotic treatments give different effects (Hu et al., 2016). Prenatal antibiotic delivery of vancomycin accelerated T1D and increased the numbers of Th17 and IFN γ ⁺ CD8⁺ T cells in the PPs, whereas neomycin treatment protected from T1D and induced tolerogenic antigen-presenting cells (Hu et al., 2016).

Early-life antibiotic use can also affect host metabolism, leading to alterations in adiposity, hepatic metabolism, metabolic hormones, and long-term development of adipose tissue, lean muscle, and bone (Cho et al., 2012; Cox et al., 2014) or accelerating body weight and bone growth (Nobel et al., 2015). However, in these studies, it is not clear whether this was mediated through alterations in the intestinal immune landscape or directly through altered microbial metabolites.

Although changes during the neonatal window are induced most profoundly by microbial changes, alterations in diet during this critical window can also change the intestinal immune landscape and affect health. Vitamin A supplementation in neonatal mice can promote early induction of oral tolerance by decreasing gut permeability and increasing Th1 cell responses (Turfkruyer et al., 2016). Given that maternal retinoid supplementation can also increase GALT induction in utero (van de Pavert et al., 2014) and maternal dietary supplementation with AhR ligands can shape intestinal ILC3 populations (Gomez de Agüero et al., 2016), the maternal diet could play a strong role in setting the stage for proper development of the intestinal immune landscape.

A similar critical window in early life in humans is also likely, although identifying the mechanisms involved is more difficult. The intestinal immune landscape in humans also undergoes dynamic developmental changes in the first few months after birth and could reflect a critical window for immune imprinting that has life-long effects. Although the intestinal microbial community does not stabilize in humans until about 3 years of age (Yassour et al., 2016; Yatsunenko et al., 2012), the precise age of the critical window in infants is not yet clear. Antibiotic use during the prenatal period or the first years of life is associated with an increased risk of asthma, allergic disease, and atopic dermatitis (Marra et al., 2009; Martel et al., 2009; Murk et al., 2011; Raciborski et al., 2012; Risnes et al., 2011; Stensballe et al., 2013). The microbiome is also implicated in susceptibility to T1D (for a review, see Paun et al., 2017). Changes in the microbiome in T1D can precede the onset of T1D (Kostic et al., 2015), and infants at risk of developing T1D have been found to harbor a distinct LPS that inhibits immune responsiveness (Vatanen et al., 2016), highlighting a potential role for the intestinal immune landscape in early life. Whether these altered disease

susceptibilities were due to changes in the intestinal immune landscape that then affected disease phenotypes at systemic sites is difficult to assess in humans. It is tempting to speculate that alterations in intestinal T cell phenotypes through antigen-specific recognition of bacterial components might play a role in setting the threshold for disease susceptibility (Tai et al., 2016).

Concluding Remarks

The intestinal immune system shows remarkable heterogeneity along the length of the gut, reflecting regional differences in intestinal function and site-specific intrinsic and environmental factors. Alterations in the development of the intestinal immune landscape, especially during a neonatal window, can have far-reaching effects and could affect susceptibility to multiple diseases later in life. Such changes can be mediated by antibiotics in early life, which alters the intestinal microbiota and leads to improper or insufficient immune development, or through dietary changes that act either indirectly through alterations in the microbiota or microbial metabolism (Tan et al., 2016a; Trompette et al., 2014) or directly through an impact on immune cells. Collectively, the findings described above suggest that specific alterations in diet and/or antibiotic usage could provide novel therapeutic opportunities for reducing disease susceptibility or progression. Before such possibilities can be realized, further mechanistic studies are required for understanding the full impact of environmental conditioning on mucosal and systemic immune system development and functionality. For example, a direct role for RA or AhR ligands on different cells types and at different intestinal sites requires clarification. Additionally, state-of-the-art mapping of human intestinal immune compartments under different conditions and ages is necessary for fully realizing the translational possibilities of these findings.

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